PHYSIOLOGY OF THE RESPIRATORY SYSTEM

NO. 628

THE COUNCIL FOR TOBACCO RESEARCH - U.S.A.

633 THIRD AVENUE
NEW YORK, N. Y. 10017

COMMITTEE:
Dr. Sommers, Chm.
Dr. Loosli
Dr. Reimann
Dr. Bing

Approved

Application For Research Grant

Date: August 10, 1967

1. Name of Investigator(s): (include Title and Degrees)
Theodore N. Finley, M.D., Associate Professor of Medicine
Aaron J. Ladman, Ph.D., Professor and Chairman, Department of Anatomy

2. Institution & Address:
we enc.
The University of New Mexico School of Medicine
915 Stanford Avenue N.E.
Albuquerque, New Mexico 87106

3. Short Title of Project:
Effect of cigarette smoking on lipids and morphology of alveolar lining material and macrophages.

4. Proposed Starting Date:
January 1, 1968

5. Anticipated Duration of this Specific Study:
3 years to December 31, 1970

6. Brief Descripton of Objectives or Specific Aims:
Four studies will be undertaken:

(a) Determination of the effect of cigarette smoking on the lipids and morphology of alveolar lining material and macrophages recovered by saline lavage of smoking and non-smoking humans.

(b) Determination of acute and chronic effects of smoking on lipids and morphology of dog alveolar lining material and macrophages in situ as well as on fractions recovered by saline lavage.

(c) Determination of the effect of cigarette smoking on the incorporation of radioactive lipids into alveolar lining material and macrophages of the dog.

(d) Determination of the origin of the alveolar macrophage by labelling circulating white cells with radioactive lipids and amino acids and monitoring the transport of these labelled cells into the alveoli of the lung by radioautographic microscopy.

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7. Give a Brief Statement of your Working Hypothesis:
(See attached sheet)

Source: https://www.industrydocuments.ucsf.edu/docs/kxwm0000

8. Details of Experimental Design and Procedures: (Attach Separate Pages)

See Attached Pages

9. Physical Facilities Available (Where Other than Administering Organization Indicate Geographical Location)

See Attached Pages

10. Additional Requirements:

See Attached Pages

11. Biographical sketches of all principal and professional personnel (append)

See Attached Pages

12. List of publications: (Five most recent as pertinent) (append)

See Attached Pages

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7. WORKING HYPOTHESIS

Preliminary experiments have shown morphologic differences in the alveolar macrophages recovered by saline lavage of smokers when compared to non-smokers. The alveolar macrophages of smokers contain an increased number of inclusion bodies of various types. In addition, the fatty acid distribution patterns of the phospholipids extracted from both the cellular and non-cellular fractions of the lavage differ in smokers and non-smokers. There appear to be more unsaturated long chain fatty acids in the phospholipids of smokers. More studies are necessary to confirm these observations.

The incorporation of labelled lipids and their precursors in alveolar macrophages of control dogs and dogs exposed to cigarette smoke will be studied to determine altered lipid pathways. We feel the above studies are necessary before more detailed studies of alterations in lung morphology and lipid metabolism in patients with various lung diseases are undertaken.

In addition, studies to determine the origin of the alveolar macrophage will be attempted because smoking may alter not only the quality but also the quantity of these cells.

8. EXPERIMENTAL DESIGN AND PROCEDURES:

Surface activity of the acellular alveolar lining material will be measured on a modified Wilhelmy balance.

Lipids will be extracted from the cellular and acellular alveolar lining material by several Folch washes using $CHCl_3$:MeOH:H_2O. The various classes of lipids (neutral and phospholipids) will be separated by thin layer chromatography using silica gel H and $CHCl_3$:MeOH:acetic acid for phospholipids and petroleum ether:diethyl ether:acetic acid for the neutral lipids. The lipids will be localized on the thin layer plates with 2,7 dichlorofluorescein and removed by scraping. Fatty acid methylesters will be formed by refluxing in MeOH and H_2SO_4 and will then be separated by gas liquid chromatography at 210° using a butanediol succinate column.

The lavage sediment containing alveolar lining material and cells will be centrifuged at 10,000 x G at 0° C for 20 minutes, fixed in cold 2.5% glutaraldehyde buffered with 0.15 M Na cacodylate to pH 7.2 for two hours, washed several times in buffer, fixed in cold phosphate buffered 1% OsO_4 for two hours, rapidly dehydrated in ethanol and then embedded in Epon 812. Sections for light and electron microscopy will be made from the epon blocks.

In attempts to isolate relatively pure monocytes from circulating blood, buffy coats from dog blood obtained by exsanguination will be processed by an electronic separator so that monocytes can be separated. They will be labelled with radioactive substances and injected into dogs. Their course into the alveolar spaces will be followed by appropriate radioautographic microscopy.

The experimental design will employ the procedures outlined in the first three paragraphs above, on humans who are smokers; non-smokers will serve as controls. (The appended reprints contain further information about our methods and observations and are included as an aide for the reviewer)

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9. PHYSICAL FACILITIES AVAILABLE

The Department of Anatomy has approximately 4,500 square feet of research space which is available for use in support of the outlined program. This includes three electron microscopes, four ultramicrotomes, two fully equipped photographic darkrooms with the supporting equipment to do excellent photomicrography. Suitable supporting technical and secretarial personnel are currently employed to assist in the implementation of the program.

Members of the Department of Medicine have laboratory areas within the medical complex. Dr. Finley's laboratory is composed of 1000 square feet and has the equipment and facilities to accomplish the biochemical procedures as outlined.

Sufficient animal quarters are present to provide adequate space to house and maintain the needed supplies of dogs.

Rooms are available at the Bernalillo-County-Indian Hospital and Albuquerque Veteran's Administration Hospital, the University's teaching hospitals, to perform the lung lavages on human subjects. X-ray and fluoroscopic equipment are at hand to monitor the positioning of the intraalveolar catheter.

10. ADDITIONAL REQUIREMENTS

We are attempting to survey a sufficiently large number of humans so that a base-line for the non-smoker and the smoker can be established. So far, over fifty subjects have undergone saline lavage. In the past, we have paid $25.00 to each individual who has volunteered for the lung lavage procedure. In the event an untoward reaction should occur, the hospital will undertake the maintenance and care of the patient. Additional funds to cover such added costs should be budgeted so that effective patient-care can be provided.

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R: REDACTED MATERIAL

CURRICULUM VITAE

Theodore N. Finley, M. D.

DATE OF BIRTH: R R

PLACE OF BIRTH: Seattle, Washington

EDUCATION: University of Washington, 1944-1950
Degree: B. S. (Chemistry)

Medical: Johns Hopkins Medical School, 1950-1954
Degree: M. D.

Internship: San Francisco County Hospital, 1954-1955

Residency: University of California Medical Center, 1955-1956
Assistant Resident in Medicine

San Francisco County Hospital, 1956-1957
Assistant Resident in Medicine

POSITIONS: Research Fellow, 1957-1958
University of Buffalo
Department of Physiology

Clinical Instructor in Medicine, 1958-1961
Cardiovascular Research Institute
University of California Medical Center

Associate Staff Member, 1960-1961
Cardiovascular Research Institute
University of California Medical Center

American Trudeau Society Research Fellow, 1958-1961

Assistant Professor of Anesthesiology, Physiology and Biophysics; Director of Anesthesiology Research University of Washington School of Medicine 1961; Associate Professor, July 1964

Associate Professor of Medicine, University of New Mexico School of Medicine, September 1964 to present.

SOCIETIES: American Physiological Society
Western Society for Clinical Research
American Society of Clinical Investigation
American Federation for Clinical Research

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BIBLIOGRAPHY - T. N. Finley, M. D.

1. Morgan, Thomas E., T. N. Finley, Gary L. Huber, and Helen Fialkow. Alterations in Pulmonary Surface Active Lipids during Exposure to Increased Oxygen Tension. J. Clin. Invest. 44:1737, 1965.

2. Morgan, Thomas E., Theodore N. Finley and Helen Fialkow. Comparison of the Composition and Surface Activity of "Alveolar" and Whole Lung Lipids in the dog. Biochim. Biophys. Acta, 106:403-413, 1965.

3. Huber, G. L., Edmunds, L. H. and Finley, T. N. Acute effects of saline lavage on pulmonary mechanics and morphology. J. Clin. Invest. (In Press)

4. Finley, T. N., Swenson, E. W., Curran, W. S., Huber, G. L. and Ladman, A. J. Bronchopulmonary lavage in normal subjects and patients with obstructive lung disease. Ann. Int. Med. 66:651, 1967.

5. Finley, T. N., Ladman, A. J., Brewer, L., and McKay, M. B. A morphological and lipid analysis of the alveolar lining material in the dog. J. Lipid Res. (In Press)

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R: REDACTED MATERIAL

CURRICULUM VITAE

Aaron J. Ladman, Ph. D.

DATE OF BIRTH: R R

PLACE OF BIRTH: Jamaica, New York, U. S. A.

EDUCATION:
New York University, New York, N. Y. A.B., 1947
Indiana University, Bloomington, Ind. Ph. D., 1952

HONORS:
American Cancer Soc., Postdoctoral Research Fellow 1952-1955;
Special Research Fellow of U.S.P.H.S. 1955-1957;
Career Development Award 1962-64;
Visiting Summer Investigator, Jackson Laboratory, Bar Harbor, Maine 1949-1951, 1953, 1954, 1956, 1957, 1959;
Fellow of American Association for the Advancement of Science, 1966.

MAJOR RESEARCH INTEREST: Functional Cytology and Cytochemistry

RESEARCH AND PROFESSIONAL EXPERIENCE:
Research Assistant, Jackson Laboratory, Bar Harbor Maine, 1947-1948;
Teaching Fellow in Anatomy, University of Cincinnati, Department of Anatomy, Cincinnati, Ohio, 1949-1949;
Teaching Fellow in Anatomy, Indiana University Department of Anatomy, Bloomington, Indiana, 1949-1952;
Research Fellow in Anatomy, Harvard Medical School, Department of Anatomy, Boston, Mass., 1952-1955;
Associate-in-Anatomy, Harvard Medical School, Department of Anatomy, Boston, Mass., 1955-1961;
Associate Professor of Anatomy, University of Tennessee Medical Units, Memphis, Tennessee, 1961-1964;
Professor of Anatomy, Chairman, Department of Anatomy, The University of New Mexico School of Medicine, 1964 to present.

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BIBLIOGRAPHY - A. J. LADMAN, Ph.D.

1. Ladman, A.J. and Finley, T.N. Electron microscopic observations of pulmonary surfactant and the cells which produce it. Anat. Rec. 154:372,1966. (Abstract)

2. Finley, T.N., Swenson, E.W., Curran, W.S., Huber, G.L. and Ladman, A.J. Bronchopulmonary Lavage in Normal Subjects and Patients with Obstructive Lung Disease. Ann. Int. Med. 66:651,1967.

3. Ladman, A.J. The fine structure of the ductuli efferentes of the opossum. Anat. Rec. 157:559,1967.

4. Finley, T.N., Conley, G.R., Huber, G.L. and Ladman, A.J. Lipid Composition of human alveolar lung material and cells. Clin. Res. 14:365,1966. (Abstract)

5. Ladman, A.J., Pratt, S.A., and Finley, T.N. Exfoliative cytology of the lung alveolus: Preliminary electron microscopic observations on cells obtained in vivo from human lungs. Elec. Micr. Soc. Am. 1967. (In Press)

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13. Budget: (1st year)

A. Salaries (Personnel by names)	% time	Amount
Professional		
Dr. Susan A. Pratt - electron microscopist	100.	R R
Technical		
Laboratory Technician	100	R
Secretarial assistance	25	
Sub-Total		R
B. Consumable Supplies (list by categories)		
Chemicals	500	
Glassware	500	
Reagents	500	
EM supplies	1,000	
Sub-Total		$2,500
C. Other Expenses (itemize)		
Animals, maintenance & procurement	1,000	
1/2 service contract for EM	700	
Consulting fee to Dr. Swenson	500	
Patient reimbursements	3,000	
Sub-Total		$5,200
D. Permanent Equipment (itemize)		
E. Overhead (15% of A+B+C)		$3,405
Total		$26,105

Estimated Future Requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Overhead	Total
Year 2	R	2,500	4,200		3,368	25,818
Year 3	R	2,500	4,200		3,495	26,795

It is understood that the applicant and institutional officers in applying for a grant have read and found acceptable the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Signature [redacted] Date
Director of Project
Telephone

Signature [redacted]
Business Officer of the Institution
Telephone

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4.

Other Sources of Financial Support

List financial support for research from all sources, including own institution, for this and/or related research projects.

Current

Title of Project	Source	Amount	Duration
Fine structure of tissues during transport behavior	National Institutes of Health Grant #1 R01 GM14435-01	$38,435	9-1-66- 8-31-67
ibid.	ibid. Grant #1 R01 GM14435-02	$28,676	9-1-67- 8-31-68
Respiratory Distress - Role of Lung & Blood Lipids	National Institutes of Health Grant #5 R01 HE09491-04	$28,500	9-1-67- 8-31-68

Pending

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